EPITHELIAL CHANGES PRODUCED BY IRRITATION.

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EPITHELIAL CHANGES PRODUCED BY IRRITATION.[1]

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(Plates VIII. to X.)

Recent investigations into the minute anatomy of cancerous growths have proved to demonstration our ignorance of the changes to which epithelial cells are liable as a result of irritation. Appearances which may easily be proved to be due to simple irritation have been taken repeatedly to represent protozoa or parasitic growths in cells. Fantastic theories about the origin of cancer have been built upon these erroneous foundations, and the more ignorant the observer, the more he has insisted upon the correctness of his interpretation, until the truth of Goethe's maxim is brought home to us with increased emphasis that "Es ist nichts schrecklicher als eine thätige Unwissenheit"[2]

The present theory that cancer is associated causally with a protozoon, only a portion of whose life cycle is spent within the cells, has done much good whether or no it ultimately be proved correct. It has led us to examine epithelial cells with the greatest care and by the most exact methods. An insight has been gained by these means into some of the changes which take place in an epithelial cell in the course of its existence, so that what was a short time since only known to one or two of the most expert histologists in Europe is now rapidly becoming a matter of common knowledge to every teacher of the microscopic art.

The investigations leading to the results with which the present paper deals were commenced with the idea of preparing a suitable nidus for the hypothetical protozoon of cancer. In pursuit of this object it became necessary to examine the cells in a large number of irritated epithelial surfaces, to ascertain what appearances were to be attributed to simple irritation, and what changes, if any, took place after the introduction of cancer.[3] My experiments last year were chiefly carried

[1] Read in the Section of Physiology at the Oxford Meeting of the British Association.

[2] "Max. u. Reflexionen," bd. iii.

[3] For further details see *Brit. Med. Journ.* 1893, vol. ii. p. 830, and 1894, vol. ii. p. 636.

out upon the vaginal mucous membranes of rabbits and of rats; this year some of the experiments have been repeated, the conjunctiva being substituted for the vagina, as it was necessary to discover whether the effects produced by irritation varied with the situation of the epithelium. The effects of irritating the vaginal epithelium may be summarised briefly as:—A general vacuolation of cells; various forms of œdema; cell nests or epithelial pearls; collections of leucocytes; and the spaces left after these leucocytes have migrated. These spaces must be distinguished from the somewhat similar appearances, with which I shall deal presently, produced by the disintegration and removal of individual cells.

I may say at once that in no case has it yet been possible to produce, by artificial irritation, that remarkable body which has been described with so much care by Dr. Ruffer, the distinguished alumnus of our university, who at present holds the responsible position of Director of the British Institute of Preventive Medicine. There are some bodies which resemble it superficially, but even those which do so most closely are deficient in the clean-cut and perfectly circular outline, as well as in the radial striation which is so distinguishing a feature in the true "cancer body." When portions of cancerous tissues have been introduced to the irritated epithelial surfaces it has twice happened that appearances presented themselves which seemed to be identical with the bodies described by Ruffer, and to be quite different from those resulting from simple irritation. It was possible that these appearances might be due to the introduction of epithelium to the irritated vaginal mucous membrane. Control experiments were, therefore, made upon two rabbits, the piece of cancer being replaced by a fresh cornea taken from another rabbit. Many interesting appearances were obtained, but no "cancer bodies" were observed. It appears, therefore, as if these bodies really bore some relation to cancer, although it has still to be shown that they are not merely some form of modified cell growth.

It is well known that the eye has already been used as a medium for the experimental inoculation of carcinoma, for Leber says:[1] "After the introduction of a portion of a glioma from the eye of a child into the vitreous of the eye of a rabbit the fragment gradually shrank without exciting any inflammation, and led to detachment of the retina." Similar experiments carried out by Messrs. Ballance and Shattock are detailed in the Morton Lecture on Cancer for 1894. These observers introduced fragments of sterile cancer into the anterior chamber of the eye of a rabbit; no change took place in the eye, but the fragment shrivelled. My own experiments are still in progress, so that I need not here dwell upon them; but it occurred to me that the grafting might be more successful if the eye were first brought into a state of chronic irritation. An endeavour was therefore made to procure a suitable amount of irritation by means which would prevent the introduction of

[1] "Die Entstehung der Entzündung," 1891, p. 231.

any fallacy in regard to the subsequent appearances. In one series of experiments, the operation of paracentesis corneæ was performed, on 18th January 1894, upon a healthy guinea-pig and a piece of the bulbous end of a vibrissa was pushed into its anterior chamber. The wound healed by first intention; for three days the cornea was cloudy, and there was a slight circumcorneal zone of inflammation. On 25th January the cornea was clear, the eye appeared to be perfectly normal, and the vibrissa could be seen lying upon the anterior surface of the iris. The cornea was hazy on 3rd February, and two small blood vessels ran into it from the conjunctiva. The animal was killed on the 5th of February; its eye was at once excised and put into Foà's solution. The hardening was completed in 30, 50, 70 per cent. spirit and absolute alcohol. Sections were made by the paraffin method. Photographs of some of the appearances observed in the conjunctival epithelium of the guinea-pig, and in the vaginal epithelium of the rabbit, into whose vagina the cornea was grafted, are reproduced in Plates VIII., IX., and X.

Plate VIII. Fig. 1 shows a cell presenting a more deeply staining central part surrounded by a clear zone. The cell substance outside the clear zone presents obvious radiations, apparently due to some portions of the protoplasm being more resistant than the rest.

Another form of this change is shown in Plate VIII. Fig. 2, where the central and presumably the degenerating portion of the cell is sharply defined, and lies in a clear space resembling a vacuole. The nucleus and the body of the cell remains in a healthy condition.

A similar but still more advanced stage is represented in Plate VIII. Fig. 3, where the cell degeneration appears to have assumed a "colloidal" form, which does not stain readily with hæmatoxylin.

Plate VIII. Fig. 4 shows a similar condition, differing from the previous specimens in the fact that the vacuole contains some solid and more readily staining body, perhaps a piece of chromatin, or possibly the whole body is a swollen leucocyte, so that the preparation might be made to lend support to the theory advanced by Metchnikoff,[1] that such vacuolation in a cell is due to an abundant secretion of digestive juices, and that it is analogous to the vacuolation observed in Protozoa while intracellular digestion is going on.

Plate VIII. Fig. 5 shows a similar state of vacuolation, save that here the degeneration has taken the form of a central mass surrounded by a layer of granules. In Plate VIII. Fig. 6 the granules have fused to form a circumferential layer. The process appears to be complete in Plate IX. Fig. 7, but two cells are involved in the destructive change.

Plate IX. Fig 8 commences another series of degenerative changes, taking place in epithelial cells as a result of chronic irritation. The cell protoplasm in this case has undergone necrosis, instead of becoming converted into "colloidal" substances.

[1] "Lectures on the Comparative Pathology of Inflammation," translated by F. H. and E. H. Starling, M.D. London, 1893.

Plate IX. Fig. 9 shows that the necrosis which commenced in the neighbourhood of the nucleus has extended towards the periphery, though the whole cell is not yet affected. It is worthy of note, perhaps, that this change is most frequent near the large granular cells, staining red with Ehrlich's hæmatoxylin, which so closely resemble the formative cells found in the segmentation cavity and in the yolk of an impregnated fowl's egg. These cells are probably eosinophile leucocytes. They are extremely numerous in the irritated conjunctival epithelium of the rabbit and guinea-pig. They are found only in the epithelial layers, however, so far as my observations extend at present, and never in the subjacent corium.

The cells are farther degenerated in Plate IX. Fig. 10, though they are not yet involved in irretrievable ruin, as the nucleus and a layer of healthy protoplasm still surround the granular mass.

Plate IX. Fig. 11 shows that epithelial cells of the squamous type possess the power of ingesting other cells. One of the most superficial cells of the conjunctival epithelium has engulfed one of the smaller red blood corpuscles. The microcyte maintains its circular shape, and is enclosed in a vacuole.

This process is still more easily seen in Plate IX. Fig. 12, where there is an amœboid body enclosed in a well-defined vacuole, within a cell of the conjunctival epithelium of a guinea-pig, in whose anterior chamber a vibrissa lay from 18th January to 5th February 1894. The enclosed body appears to have a slightly yellowish tinge, due perhaps to hæmoglobin, but it is, I believe, a leucocyte which has gained admission to the cell. It does not correspond to any of the forms of paranucleus or *nebenkern*, described by Gaule, Platner, Heidenhain, or other observers, but it is exactly similar to an appearance represented by Professor Pawlowsky,[1] and described by him as a parasite, since it was seen in a malignant growth.

A double inclusion has taken place in Plate X. Fig. 13, which is thus a more complex example of epithelial ingestion. It represents a cell containing another cell within it, lying in a vacuole. The including cell itself has a microcyte in its substance, also enclosed in a vacuole. The included as well as the including cells are undergoing degenerative changes.

Plate X. Fig. 14 is another good instance of cell inclusion occurring in surface epithelium. The including cell contains a large vacuole, and within it is a leucocyte. A farther stage of the same process appears to be represented in Plate X. Fig. 15, where two leucocytes are enclosed in the space left by the degeneration of one or more epithelial cells whose horny remains bound the space with a jagged outline. This appearance is easily distinguishable from the somewhat similar space seen in the *Brit. Med. Journ.* 1893, vol. ii. p. 832, fig. 9, where the outline is smooth and the leucocytes are much more numerous.

Plate X. Figs. 16 and 17 belong to another group of cases, for they

[1] *Virchow's Archiv*, 1893, bd. cxxxiii. plate xiii. fig. 28.

represent appearances seen after the introduction of scirrhus into the irritated vagina of a rabbit. They are probably only the results of cell degeneration, but the remarkably clean-cut outline of the cell in Plate X. Fig. 17 seems to bring it somewhat nearer to the "cancer bodies." It resembles the appearance drawn by Professor Pawlowsky.[1]

Plate X. Fig. 18 shows the appearance met with in the epithelium of a rabbit's vagina a few days after the introduction of a piece of an epithelioma. This figure is an enlargement of fig. 10, published by me in the *Brit. Med. Journ.* last year. The negative is absolutely untouched, but the enlargement has brought into view the radial striation which was before invisible, so that the body still more closely resembles those described by Dr. Ruffer.

I have already called attention elsewhere[2] to the interesting points of correspondence between Malaria and Carcinoma from a theoretical standpoint. The monographs on Malaria by Marchiafava and Bignami and by Mannaberg have recently been translated into English, and published by the New Sydenham Society, and in this volume will be found a remarkable confirmation of the views I have set out. A comparison of plate i. figs. 28, 29, and 30 in Marchiafava's work and plate ii. figs. 6–10 in Mannaberg's monograph, will show how closely some forms of the malarial parasite approximate the appearances observed by Dr. Ruffer in cancer, and by myself after grafting cancer upon irritated epithelium. The appearances are so similar in the two classes of cases that Marchiafava's description of the parasite in the "summer-autumn tertian" fever applies equally well to the intracellular conditions met with after grafting carcinoma. He says (p. 57): "The phase of the young forms is represented by hyaline plasmodia, without pigment, diaphanous, generally rather large, in size from a fifth to a fourth of that of a red blood corpusele; there may also be found, along with these, amœbæ of very small dimensions, not larger than a third of the former. These forms, which, as in the quotidian, may be annular and discoid in shape, or display lively movements, are contained, for the most part, in red blood corpuscles of normal aspect." Read epithelial cells for red blood corpuseles, and the description is accurately adapted to the appearances which I have already described,[3] and which are reproduced in Plate X. Fig. 18, where it will be seen that the "cancer bodies" vary in size, and that, though two are intracellular, the third lies between the cells as if it had been derived from some extraneous source.

The interest of the present observations lies in the explanation they afford of analogous forms frequently seen in malignant tumours. It is obvious that if similar appearances are not unusual in normal tissues, or in tissues which have only been subjected to slight irritation, they cannot be considered as parasitic when they are met with in cancer or sarcoma. We must become expert histologists before any decided advance can be

[1] *Op. cit.* plate xiii. fig. 18.

[2] *Brit. Med. Journ.* 1894, vol. ii. p. 636.

[3] *Ibid.* 1893, vol. ii. pp. 833, 834.

made in our knowledge as to whether cancer is due to a micro-organism or not, for we must be perfectly familiar with the various appearances met with in epithelium which is either normal or is only slightly removed from a healthy condition. The readiest method of doing this seems to be by a prolonged and careful study of epithelial surfaces which have been brought into the condition in which cancer is known to occur clinically; that is to say, with decadent cells and in a state of chronic irritation. When this has been done and done thoroughly we shall be at liberty to look about and to ascertain whether there are any appearances peculiar to cancer, which are not the result of simple irritation. We shall be on the high road to a successful termination of our quest as soon as these are found. Ruffer's bodies at present appear to be instances of such a difference, because they have not yet been found as a result of chronic irritation, though they occur when cancerous growths are brought into contact with irritated cells. We ought, therefore, to use our best endeavours to work out the life-history of these bodies. If they fail us, and prove after all to be only modified cells, we should turn without feeling disheartened to the next most likely form, for it is only by a process of exclusion that we can arrive at the truth. The task appears to be well-nigh endless, for it seems as if the epithelia of different animals had markedly different properties; thus in rabbits degenerative changes are common after irritation, whilst in guinea-pigs cell inclusions seem to be the more frequent result.

I have refrained in this paper from advancing any theories as to the appearances observed, and have contented myself with recording what seem to be facts. The question of phagocytosis by epithelial cells is an exceedingly tempting one to discuss, but at the present time our knowledge of the origin of cancer is more likely to be promoted by careful and correct observation than by adducing new theories. The excellent critical digest by Professor Strœbe, which appeared in Ziegler's *Centralbl. f. allg. Path. u. path. Anat.*, at the beginning of the year, gives no less than 112 references to the work of various observers published from 1890–1893, and even this list is not exhaustive. All these papers are upon the sporozoal causation of cancer. It is therefore of the utmost importance that the foundations upon which such a superstructure has been raised should be examined with the utmost care, lest any flaw therein should lead to its complete destruction.

The explanations I have felt entitled to offer are merely tentative. Few things are more difficult than to describe accurately minute histological changes, and even to determine whether a body is in a cell, on a cell, or below a cell. I have endeavoured, however, by means of photographs to portray the cells as they appear to me at the present time, for there is always in my mind that saying of John Hunter, our great master in the art of observation, "Never ask me what I have said or what I have written; but if you will ask me what my present opinions are, I will tell you."

DESCRIPTION OF PLATES VIII. TO X.

FIGS. 1, 2.—Conjunctival epithelium of guinea-pig. Vibrissa in anterior chamber, January 18th to February 5th, 1894. Foà and Ehrlich's hæmatoxylin. (× 600.)

FIGS. 3–7.—Vaginal epithelium of old doe white rabbit, iodined September 22nd to November 7th, 1893. Cornea of healthy rabbit in vagina, November 7th to 10th, 1893. Foà. Ehrlich's hæmatoxylin. (× 600.)

FIG. 8.—Vaginal epithelium of old brown rabbit, iodined October 31st, 1892, to February 16th, 1893. (× 600.)

FIGS. 9–13.—Conjunctival epithelium from same guinea-pig as Figs. 1 and 2. (× 600.)

FIG. 14.—Vaginal epithelium of same rabbit as Figs. 3–7. (× 600.)

FIG. 15.—Vaginal epithelium of same rabbit as Fig. 8. (× 600.)

FIGS. 16, 17.—Vaginal epithelium of brown rabbit iodined for six months, and from June 19th to 23rd, 1893; scirrhus introduced June 23rd; animal killed June 26th, 1893. Foà. Ranvier's hæmatoxylin. (× 600.)

FIG. 18.—Vaginal epithelium of rabbit, iodined February 13th to 16th, 1893. Secondary epitheliomatous nodule introduced February 16th; animal killed February 19th, 1893. Foà. Ehrlich's hæmatoxylin. Enlarged from photograph magnified 400 times.

FIG. 1.

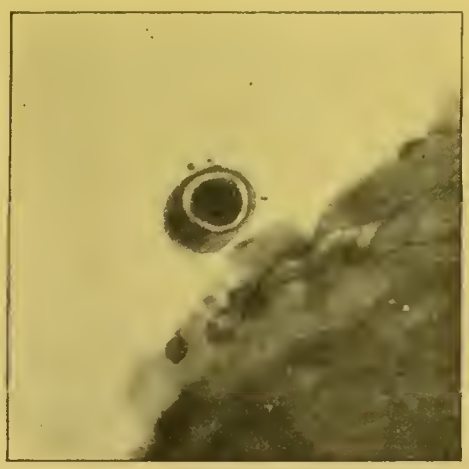


FIG. 2.

FIG. 3.

FIG. 4.

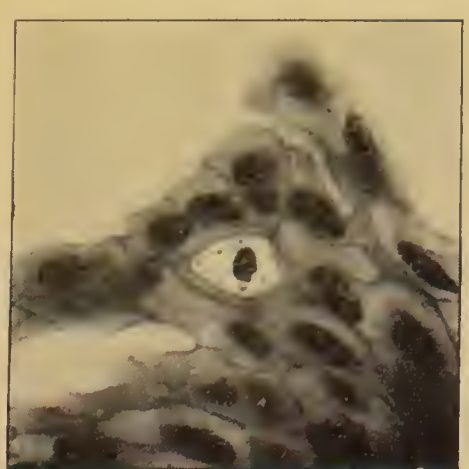


FIG. 5.

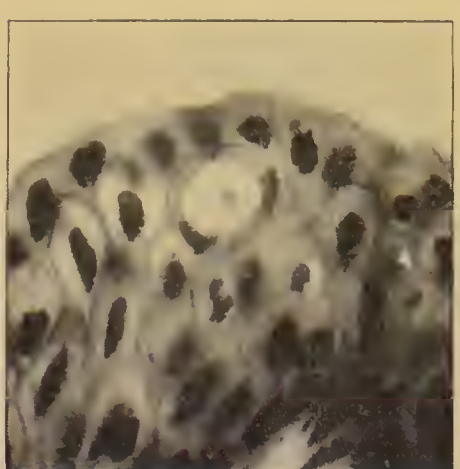
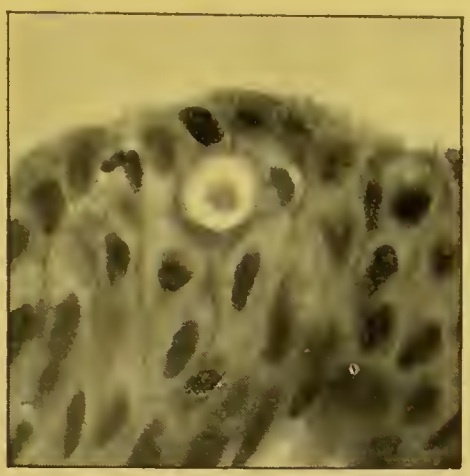


FIG. 6.



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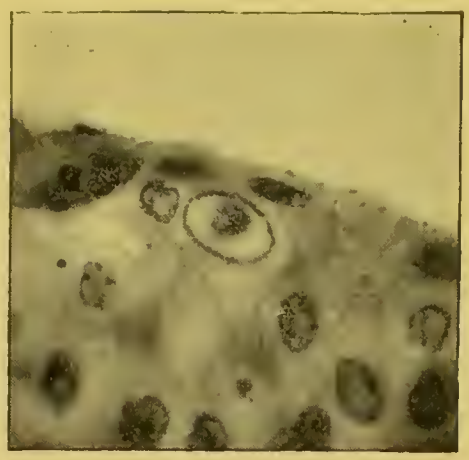

FIG. 8.

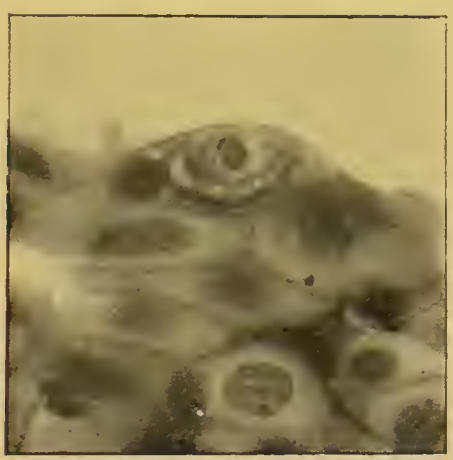

FIG. 9.

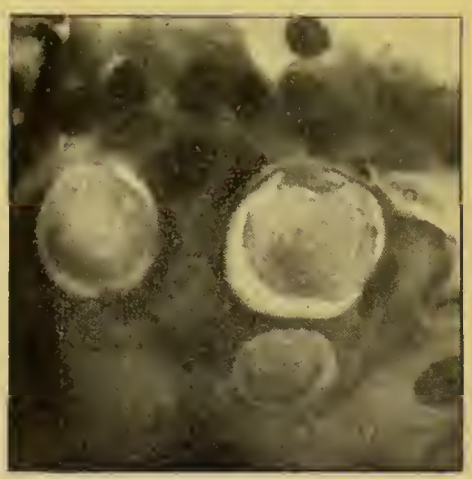

FIG. 10.

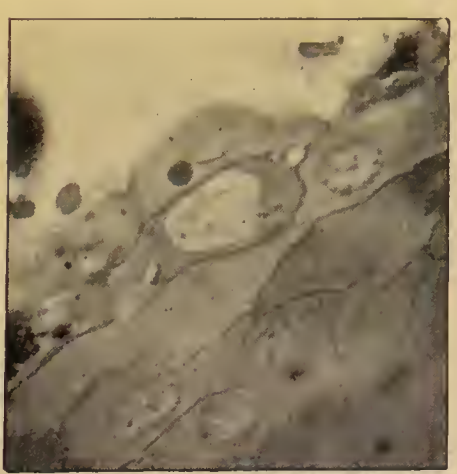

FIG. 11.

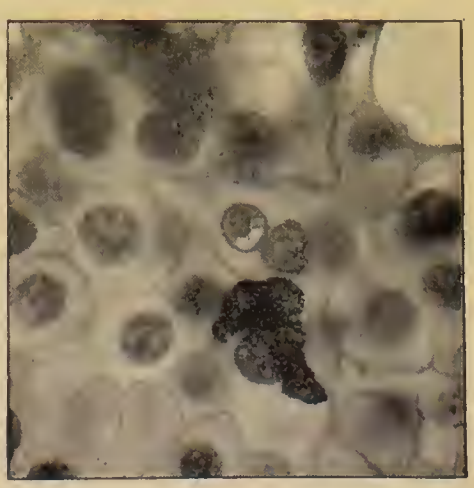

FIG. 12.

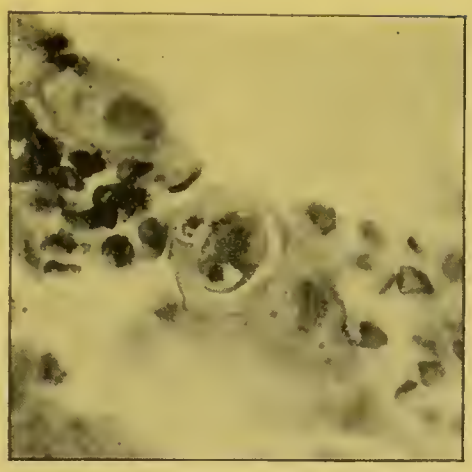

FIG. 13.

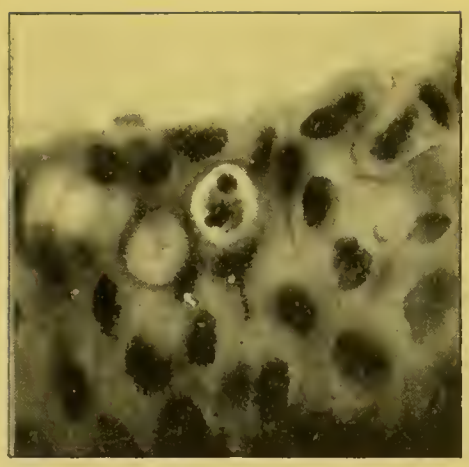

FIG. 14.

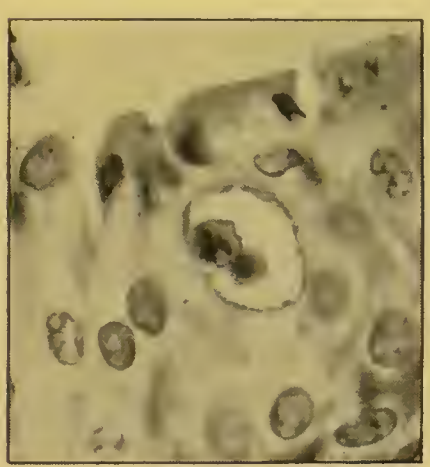

FIG. 15.

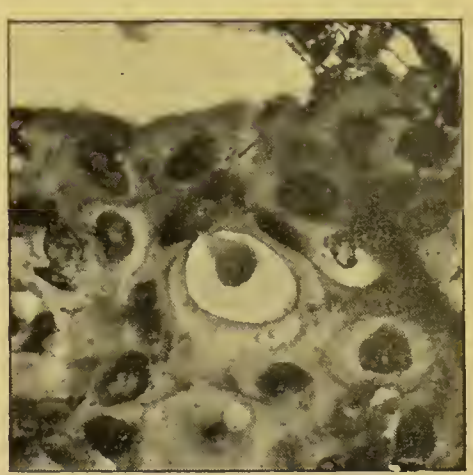

FIG. 16.

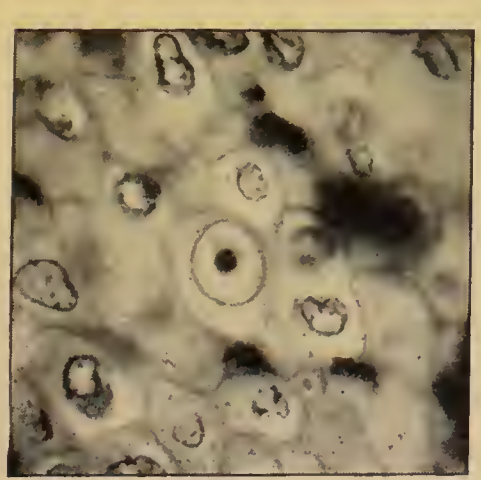

FIG. 17.

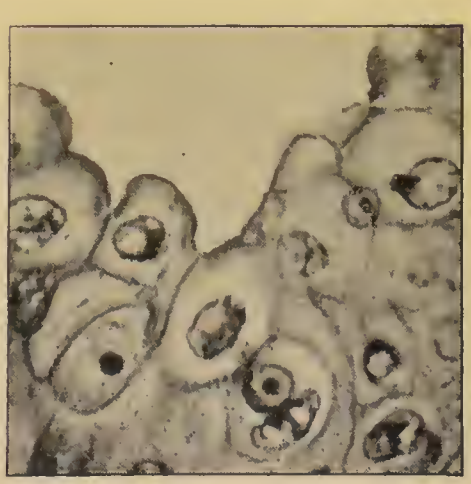

FIG. 18.